UNIVERSITY OF PENNSYLVANIA

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A THESIS

IN PHYSIOLOGY

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OXYGEN AND CARBON DIOXIDE TRANSPORT BY THE BLOOD OF THE URODELE, AMPHIUMA TRIDACTYLA

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This paper is a presentation of the oxygen dissociation curves and of the carbon dioxide absorption curves of the blood of *Amphiuma tridactyla* together with comparisons with similar curves from the literature of the carp, the turtle, the frog, and man. In particular, the applicability of Hill's equation to the bloods of these species and the shape of the oxygen dissociation curve as an adaptive mechanism are briefly discussed. In addition, the properties of the carbon dioxide absorption curves, especially of those which are a result of the low hemoglobin content, are brought out.

Technic

The blood was drawn from the heart 3–5 minutes after the injection of one cc. of 1:1000 heparin in 0.7 per cent sodium chloride to prevent clotting of the blood in the syringe during withdrawal. It was then transferred to a glass container coated with sufficient sodium fluoride and sodium oxalate to make a final concentration of about 0.1 to 0.2 per cent and kept at 2–8° C. until used.

The equilibration of the blood with the desired tensions of oxygen and of carbon dioxide, for 30 minutes at 22–26° C., was performed by a technic similar to that of Austin, Cullen, Hastings, McLean, Peters, and Van Slyke (1922). Blood gases were determined in duplicate by the method of Van Slyke and Neill (1924). Analyses of the gas phase were made by the Haldane-Henderson apparatus. Hematocrite determinations of corpuscular volume were made before and sometimes after samples were drawn for equilibration. No difficulty was encountered in the use of the ferricyanide method for *Amphiuma* blood in contrast with the experience of Krogh and Leitch (1919) with fish blood. Caprylic alcohol as an antifoamer was omitted because of clot formation.

Oxygen Consumption of Amphiuma Blood

The spontaneous diminution of the oxygen of blood so common particularly with nucleated cells is quite marked in *Amphiuma* blood. It was found (Table I) that keeping the blood at 2–5° C. except during

the period of actual equilibration practically eliminated this spontaneous oxygen consumption. The presence of physiological amounts of carbon dioxide apparently diminishes appreciably the oxygen loss. The slight loss which may occur during 20–30 minutes of equilibration and handling is negligible. Wastl (1928) has successfully used KCN to prevent oxygen consumption in the blood of the carp, but its effect in *Amphiuma* blood appeared variable and we abandoned its use in favor of cold to eliminate this source of error.

TABLE I

Effect of Temperature on the Oxygen Consumption of Amphiuma Blood

Temperature = 20° C.

Time *minutes*	Oxygen Content *vol. per cent*
0	2.64
59	1.41
118	0.20
197	0.0

Temperature = 3° C.

Time *minutes*	Oxygen Content at Varying CO_2 Tensions					
	1–3 mm.	25 mm.	42 mm.	44 mm.	53 mm.	60 mm.
0	3.49	4.64	3.00	4.57	4.52	6.23
20			2.98		4.52	6.18
60	3.16	4.70	2.64	4.41	4.46	5.94
120	2.89	4.61	2.55	4.23	4.19	
180	2.69	4.50	2.69	3.99		
240	2.69	4.59				

THE SOLUBILITY COEFFICIENT OF OXYGEN IN AMPHIUMA BLOOD

It is usually assumed as a sufficient approximation that the solubility of a gas in blood or serum, relative to its solubility in water is proportional to the water content of the blood. For *Amphiuma* blood the relative solubilities in serum and in whole blood accordingly would be about 95 per cent and 87 per cent respectively. We have measured αO_2 directly in both serum and whole blood of *Amphiuma* in the following way: Samples of serum and of whole blood were equilibrated with air and with 99.6 per cent oxygen. To diminish spontaneous oxygen consumption the equilibration was performed at 4° C. and the solubility coefficient relative to that of water at the same temperature calculated. After equilibration blood was drawn into a mercury receiver. Sampling and transfer to the Van Slyke apparatus was accomplished by using a Barcroft pipette. Assuming that the oxygen of

the air is sufficient to fully saturate the hemoglobin, αO_2 may be calculated from the difference in the oxygen content of the samples by the equation:

$$\alpha O_2 = \frac{760(V - V')}{P - P'},$$

where V and V' are respective oxygen contents in volume percentage and P and P' are respective oxygen partial pressures in mm. Hg. Table II shows that the relative solubilities of oxygen in *Amphiuma* serum and

TABLE II

Solubility Coefficient of Oxygen in Amphiuma Whole Blood and Serum

Temperature		Oxygen Tension	Oxygen	αO_2
° C. ± .5°		*mm. Hg*	*vol. per cent*	
	3	749	3.90	0.0393
		157	0.84	
	6	754	3.98	
		158	0.79	0.0408
	3	754	3.98	0.0401
Serum				
	3	748	4.04	
		157	0.83	0.0403
	4	157	0.84	0.0410
Av.	4	751	3.97	
		157	0.82	0.0403
		Average αO_2 = 92% of αO_2 water.		
	4	765	12.23	
		161	9.05	0.0400
	4	764	12.28	
		161	9.05	0.0406
Whole blood	4	740	12.90	
		156	9.77	0.0398
Av.	4	756	12.45	
		159	9.29	0.0402
		Average αO_2 = 92% of αO_2 water.		

whole blood are about 92 per cent of that in water; and this figure is used in the subsequent calculations. These figures fail to demonstrate a difference in the αO_2 between serum and whole blood of *Amphiuma,* which is not surprising in view of the low corpuscular volume of the blood.

Hemoglobin Content of the Cells

The corpuscular volume varies considerably and is slightly higher than that found by Southworth and Redfield (1926) for the turtle. The oxygen capacity also varies greatly in *Amphiuma* blood. In Table III it is seen that the ratio of oxygen capacity to cell volume is roughly

Table III

Variation in Oxygen Capacity and in Corpuscular Volume in the Blood of Amphiuma and the Ratio of Oxygen Capacity to Cell Volume

Red Cells *vol. per cent*	Oxygen Capacity *vol. per cent*	Oxygen Capacity *cell volume*
14	2.52	0.18
15	4.52	0.30
17	4.7	0.28
20	5.01	0.25
20	5.8	0.29
25	6.1	0.24
28	8.38	0.30
36	9.64*	0.27
52	9.42*	0.18

* Concentrated blood.

constant, and averages 0.25. The data of Southworth and Redfield (1926) on the turtle show an approximate ratio of oxygen capacity to cell volume of 0.50 and the average figure for human blood is said to be 0.45. The red cells of both *Amphiuma* and the turtle are nucleated and as a rough approximation of the fraction of the volume occupied by the cytoplasm we take 0.80, and calculate the concentration of hemoglobin per unit volume of cytoplasm. It appears from these data that the concentrations of hemoglobin in the cytoplasm of the red cells of *Amphiuma* and the turtle are 0.32 and 0.63 volumes of oxygen per volume of cytoplasm respectively, or 71 per cent and 140 per cent of the concentration of hemoglobin in the cytoplasm of the human red cell, an interesting divergence in this factor for the three different species.

The Oxygen Dissociation Curve

The oxygen-binding properties of both hemoglobin solutions and bloods, except at low and high degrees of saturation, may be represented with sufficient accuracy by the equation of Hill (1910):

$$\frac{HbO_2}{Hb} = K_0 P^n.$$

We have found it convenient to analyze our data on *Amphiuma* blood by means of this equation and it is evident that it holds quite well for this blood as shown by Fig. 1. Here are plotted the points of an

oxygen dissociation curve at 43 ± 3 mm. of carbon dioxide (Table IV) and for comparison, points from similar data for the carp (Wastl, 1928), turtle (Southworth and Redfield, 1926), and man (Bock, Field and Adair, 1924). The respective curves are calculated from the values of

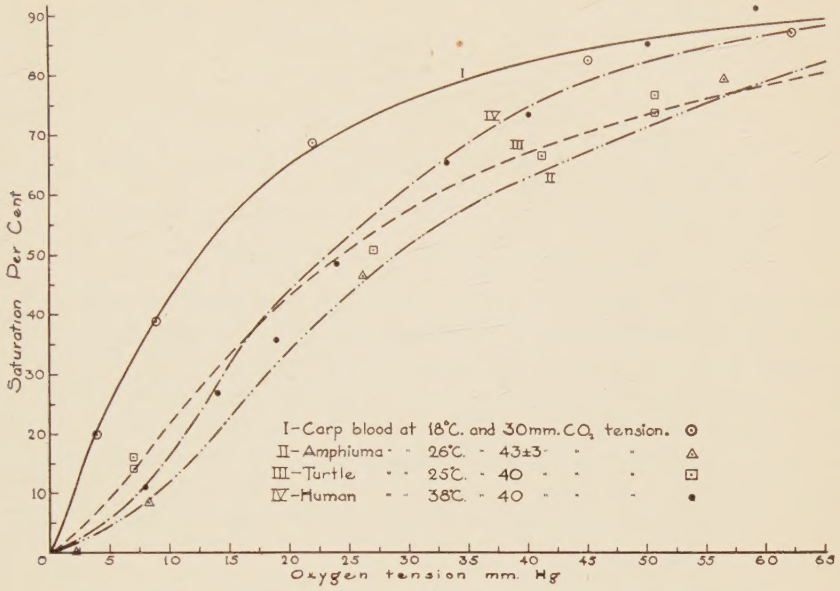
Saturation Per Cent
Oxygen tension mm. Hg
I-Carp blood at 18°C. and 30mm. CO_2 tension.
II-Amphiuma · · 26°C. · 43±3 · · ·
III-Turtle · · 25°C. · 40 · · ·
IV-Human · · 38°C. · 40 · · ·

FIG. 1. Oxygen dissociation curves. The lines are calculated by Hill's equation and plotted using the n and K_0 values (Table V) from the original data (points).

n and K_0 for the blood of each species, given in Table V. These constants in turn were obtained in the usual way by plotting log HbO_2/Hb against log PO_2, as in all cases, the points between approximately 10 to 90 per cent saturation fall quite closely on a straight line represented by the equation,

$$\log \frac{HbO_2}{Hb} = \log K_0 + n \log P,$$

from which the constants K_0 and n have been calculated. The conformity of the experimental observations (points) with the equation of Hill (lines) shows that Hill's equation holds reasonably well within the limits specified for these divergent species. *Amphiuma* resembles human blood in showing an " S " shaped oxygen dissociation curve which is absent in the carp and turtle. It is well known that the " S " shaped curve is associated with high values of N (greater than 1.8). This is apparent from the comparison of the curves of these four

TABLE IV

Oxygen Dissociation Curve of *Amphiuma* Blood. Temperature = 26° C. Carbon dioxide tension = 43 ± 3 mm. Oxygen capacity = 8.4 vol. per cent. $\alpha O_2 = 0.92\ \alpha O_2$ (in water).

	O_2 Tension	HbO_2	Saturation
	mm. Hg	*vol. per cent*	*per cent*
1.	2.2	0.01	0.0
2.	8.4	0.70	8.3
3.	15.8	2.2	26.3
4.	26.2	3.9	47.0
5.	56.4	6.7	80.0
6.	79.6	7.6	91.0
7.	143.5	8.4	100.0

forms whose n values vary from 1.4 to 2.0. The absence of the " S " shape has been interpreted by Krogh to signify an adaptation of bottom forms such as the carp to low oxygen tension, whereas the free-swimming forms, *e.g.*, the trout, whose curves are " S " shaped, do not possess this adaptation. The air-breathing *Amphiuma,* despite its habit of remaining submerged for considerable periods, appears not to possess this adaptation. That the " S " shaped curve is not a necessary property of the blood of air-breathing animals is evident from its absence in the turtle, whose n value is 1.5.

TABLE V

Hill's n and K_0 for Carp, Turtle, Man, and Amphiuma Whole Blood

Species	n	$K_0 \times 10^{-3}$	
Carp	1.3	36.0	Wastl (1928)
Amphiuma	1.8	2.5	
Turtle	1.5	8.3	Southworth and Redfield (1926)
Man	2.0	1.9	Bock, Field, and Adair (1924)

EFFECT OF CARBON DIOXIDE ON THE OXYGEN DISSOCIATION CURVE

The increased acidity of the blood with increased carbon dioxide tension has been abundantly shown to decrease the oxygen saturation at a given tension. In other words, K_0 is decreased by increasing carbon dioxide tension. That this effect is manifest in *Amphiuma* is shown by the data of Table VI. The values of K_0 have been calculated by Hill's equation, using $n = 1.8$. This decrease of the affinity of hemoglobin for oxygen with increased acidity is in accord with experience in many species such as man, dog, fishes, and turtle.

TABLE VI

Effect of Carbon Dioxide on K_0 of Amphiuma Blood

	Oxygen Capacity	CO_2 Tension	Oxygen Tension	Saturation	$K_0 10^{-3}$
	vol. per cent	*mm. Hg*	*mm. Hg*	*per cent*	
1.	5.8	*a.* 2.3	53.0	88.0	5.7
		b. 38.0	83.0	84.0	1.8
2.	4.5	*a.* 1.2	26.0	58.0	3.9
		b. 43.5	85.0	87.0	2.2
3.	2.5	*a.* 1.9	44.0	78.0	4.2
		b. 42.7	48.0	62.0	1.5

THE TRANSPORT OF CARBON DIOXIDE

Figure 2, made from the data of Table VII, shows three carbon dioxide absorption curves of oxygenated *Amphiuma* blood. Curve I is characterized by a rather low CO_2 capacity, about 39 volumes per cent at 70 mm. CO_2 tension, and also by the fact that when this blood is equilibrated with gas mixtures lacking carbon dioxide, very little of this gas remains in the blood. Curves II and III, on the other hand, are

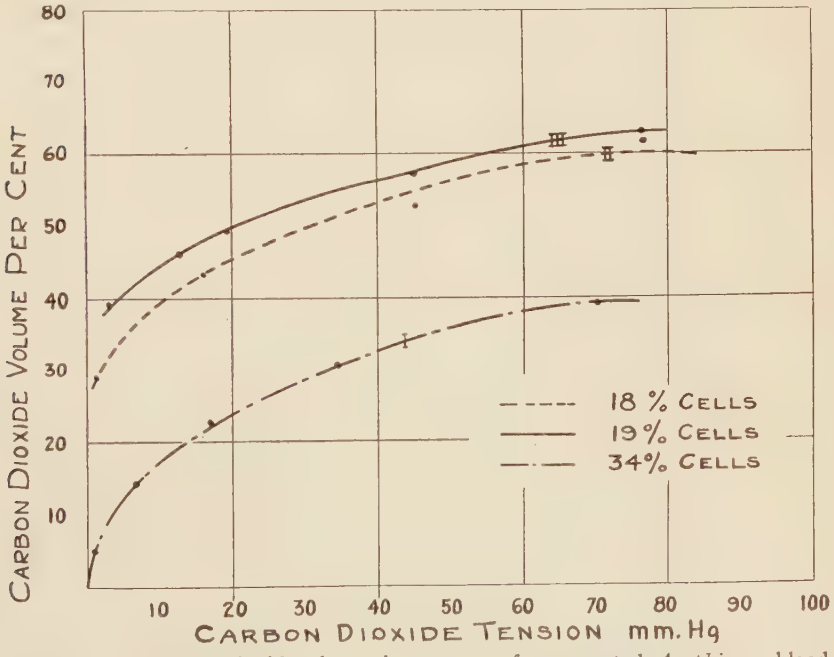

FIG. 2. Carbon dioxide absorption curves of oxygenated *Amphiuma* blood. Temperature 24 ± 2° C.

characterized by a much higher carbon dioxide capacity, approximately 63 volumes per cent at 78 mm. carbon dioxide tension, and further by the fact that when these bloods are exposed to low carbon dioxide tension during equilibration a relatively large amount of carbon dioxide, 30–40 volumes per cent, still remains in the blood. The considerable difference between curve I and curves II and III is probably due to the large variation in hemoglobin content. It is well known that hemoglobin functioning as an acid can combine with only a limited amount of base so that only limited amounts of $NaHCO_3$ will be decomposed into sodium hemoglobinate and carbon dioxide when blood which contains but a limited amount of hemoglobin is exposed to zero or low tension of carbon dioxide.

TABLE VII

Carbon Dioxide Absorption Curves. (See Fig. 4.) Temperature = 24 ± 2° C. Oxygenated blood.

Curve No.	Point No.	CO_2	CO_2
		mm. Hg	*vol. per cent*
I (Cells = 34 per cent)	1.	1.1	4.48
	2.	6.7	14.08
	3.	16.9	22.36
	4.	34.4	30.25
	5.	70.3	38.85
II (Cells = 19 per cent)	1.	3.4	39.45
	2.	12.9	46.11
	3.	19.5	48.79
	4.	45.4	56.78
	5.	76.1	62.94
III (Cells = 18 per cent)	1.	1.1	28.62
	2.	16.3	43.0
	3.	45.5	53.32
	4.	76.4	61.05

The amount of sodium bicarbonate which will be decomposed by the hemoglobin in passing from a definite tension, *e.g.* 40 mm. of carbon dioxide, to a tension of zero may be calculated as follows: let the maximum base bound per unit of hemoglobin be b_0 and the base bound at 40 mm. carbon dioxide per unit of hemoglobin be b. Then if [Hb] be the concentration of hemoglobin in the blood, the $NaHCO_3$ decomposed in the reaction—

$$NaHCO_3 + HHb \rightarrow NaHb + H_2CO_3$$

is obviously $\triangle NaHCO_3 = (b_0 - b) \cdot [Hb]$. A rough extrapolation of the curves of Hastings, Sendroy and Heidelberger (1924) for horse

hemoglobin gives $b_0 = 8$ and $b = 2$. That is, blood in passing from 40 mm. to zero millimeters of carbon dioxide tension will decompose no more than $b_0 - b$ volumes of $NaHCO_3$ for each volume per cent of oxygen capacity. For the *Amphiuma* bloods whose carbon dioxide absorption curves are shown in Fig. 2 the oxygen capacities are respectively 8.5, 4.5 and 4.5 volumes per cent. The corresponding amounts of $NaHCO_3$ decomposable are therefore 51, 27 and 27 volumes per cent. In the case of curve I, however, the amount of $NaHCO_3$ at 40 mm. was 32 volumes per cent which, being less than the maximum of 51, will be entirely decomposed at zero carbon dioxide tension. This was found to be the case as shown in Fig. 2. In the case of curves II and III, where at 40 mm. carbon dioxide tension the $NaHCO_3$ is approximately 55 volumes per cent, only 27 volumes per cent will be decomposed. This was found to be approximately the case as seen in Fig. 2. This calculation, of course, is admittedly the roughest sort of approximation from insufficient data and is offered only as a semi-quantitative explanation of the phenomenon of incomplete decomposition

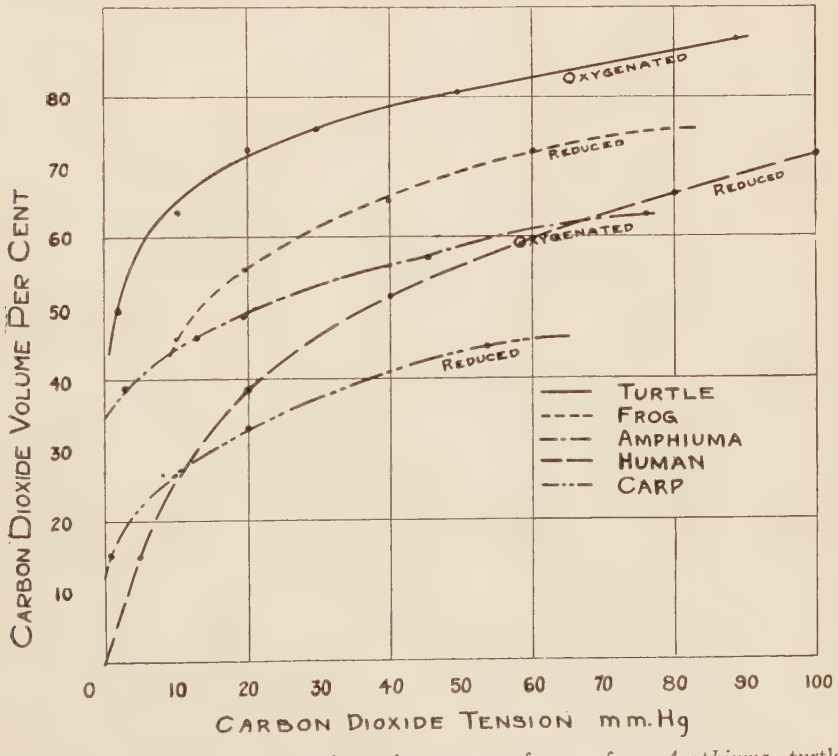

FIG. 3. Carbon dioxide absorption curves of carp, frog, *Amphiuma*, turtle and man. Temperature, human curve 38° C., all others, room temperature.

of $NaHCO_3$ by hemoglobin at zero millimeters of carbon dioxide tension. A quite similar qualitative explanation of the same phenomenon in turtle blood was first offered by Southworth and Redfield (1926).

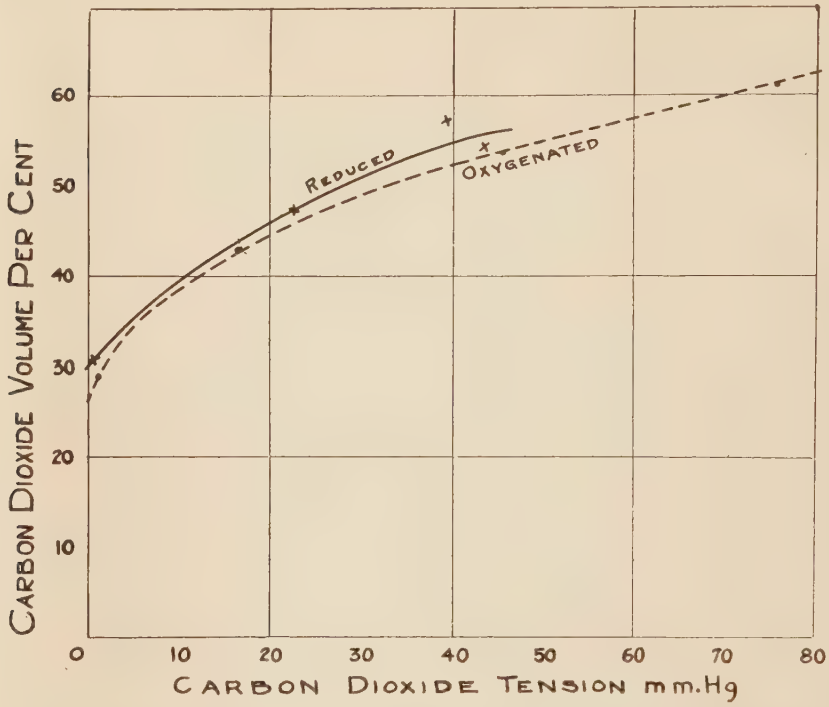

FIG. 4. The effect of oxygenation and reduction on the carbon dioxide absorption curve of *Amphiuma* blood. Eighteen per cent cells. Temperature 24° C.

Figure 3 shows a number of carbon dioxide absorption curves for the carp, frog, *Amphiuma,* turtle, and man, representative of four of the classes of vertebrates. All of the curves for the lower vertebrates resemble each other more than they do the mammalian curve. In these few examples the amphibia occupy an intermediate position with respect to carbon dioxide content between the teleostian carp and the reptilian turtle. The marked flatness of the turtle curve is attributed by Southworth and Redfield (1926) to the low corpuscular volume, *i.e.,* the low Hb of turtle blood. Wastl (1928) gives the same sort of explanation for the essentially parallel curve for carp blood. A similar explanation for the flatness of the *Amphiuma* curve is indicated just as it is for the other lower vertebrates, since the blood of all of these animals shows a corpuscular volume only about one-half to one-third that of human blood.

The Effect of Oxygenation and Reduction of the Blood on the Carbon Dioxide Transport

Figure 4 shows the difference in the amounts of carbon dioxide carried by oxygenated and reduced *Amphiuma* blood. The difference in carbon dioxide content in the two cases is not so great for *Amphiuma* blood as that found by Christiansen, Douglas, and Haldane (1914) for human blood. In fact, this difference amounts to about two volumes

Table VIII

The Effect on Carbon Dioxide Content of Oxygenation and Reduction of Amphiuma Blood. (See Fig. 4.) Temperature = 24° C.

Curve No.	Point No.	CO_2 Tension	CO_2
		mm. Hg	*vol. per cent*
I (Reduced blood, O_2 capacity 4.4 vol. per cent)	1.	0.7	30.87
	2.	22.7	47.28
	3.	39.8	56.94
	4.	43.6	54.34
II (Oxygenated blood, O_2 capacity 4.4 vol. per cent)	1.	1.1	28.62
	2.	16.3	43.0
	3.	45.5	53.32
	4.	76.4	61.05

per cent at physiological levels for *Amphiuma,* while for man the corresponding value is about 5.5 volumes per cent. If the difference in the amounts of carbon dioxide carried by oxygenated and by reduced blood be divided by the oxygen capacity of the sample of blood, a ratio $\frac{\triangle CO_2}{\triangle O_2}$, *i.e.,* the increase of carbon dioxide content per unit of oxygen capacity, is obtained. The value of this ratio in the case of human blood is about 0.28 volume of carbon dioxide per unit volume of oxygen capacity. In five experiments we attempted to determine this ratio closely, but our results were quite divergent. We found for *Amphiuma* blood values of $\frac{\triangle CO_2}{\triangle O_2}$ as follows: 0.23, 0.31, 0.46, 0.71, and 0.93, averaging 0.54. It is obvious that the calculation of this ratio is subject to considerable error since it is the ratio of small differences of large volume; nevertheless, the results are all in the same direction as in the blood of man. The mean value of the ratio for *Amphiuma* is, of course, very approximate. Physiologically, however, oxygenation and reduction have little effect on the transport of carbon dioxide by *Amphiuma* blood. In this respect, also, the *Amphiuma* is like the turtle. The data for the curves of Fig. 4 are included in Table VIII.

Summary

1. The corpuscular volume of *Amphiuma* blood varies considerably, from 12 to 35 per cent.

2. The oxygen capacity varies from 3 to 10 volumes per cent.

3. It is shown that the oxygen dissociation curve is like the typical mammalian curve with certain features in common with those of the turtle and carp.

4. The presence of physiological amounts of carbon dioxide affects the oxygen dissociation curve in the usual way.

5. The comparative values of n and K_0 of the Hill equation are given for the blood of *Amphiuma,* carp, turtle and man, and the equation of Hill for these bloods is shown to hold within the limits specified.

6. The mechanism for the transport of carbon dioxide in the *Amphiuma* blood is much like that in the turtle and the flatness of the carbon dioxide absorption curve is explained as a function of the limited amount of hemoglobin.

7. The difference in carbon dioxide carried by oxygenated and reduced blood is quite small and probably has little physiological significance, though the increase in carbon dioxide content per unit of oxygen capacity is in the same direction as that for man.

The major portion of this work has been carried out under the direction of Dr. William C. Stadie. I wish to thank him for his interest in this problem and for his continual aid and encouragement.

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